SnaBI
SphI
HindIII
aat2
(Bam/BglII)
EcoRI

psport T5 #3

8.3.kb

amp

ClaI

NdeI

BamHI
xbaI
sst
salI
EcoRI
Kpn
PstI

lacI

lacP

Fig. 1

tac

pTTQ. Taq

amp

lacI^Q

Kpn I

Xho I

BamH I

BamH I

Xba

Sal I

Sph I

Hind III

Fig. 2

Fig. 3

The region contains the O-helix homologous sequences.

The polymerase

pUC-Tne

pSport-Tne

pUC-Tne restriction sites (top to bottom): HindⅢ, BamHI, SpeI, BamHI, SphI, EcoRV, EcoRV, SstI

pSport-Tne restriction sites (top to bottom): SphI, HindⅢ, BamHI, SpeI, BamHI, SphI, EcoRV, EcoRV, SstI, BamHI

* * * S E Q U E N C E * * *

BamHI

```
 1  GGATCCAGAC TGGTGGATCG TCAGTGCGGA TTATTCCCAA ATAGAACTCA GAATCCTCGC
     G  S  R   L  V  D    R  Q  C  G   L  F  P  N   R  T  Q   N  P  R
→  D  P  D   W  W  I    V  S  A  D   Y  S  Q  I   E  L  R   I  L
     I  Q   T  G  G    S  V  R   I  I  P  K   -  N  S   E  S  S

61  TCATCTCAGT GGTGATGAGA ACCTTGTGAA GGCCTTCGAG GAGGGCATCG ATGTGCACAC
     S  S  Q   W  -  -    E  P  C  E   G  L  R   G  G  H   R  C  A  H
→  A  H  L   S  G  D  E   N  L  V   K  A  F  E   E  G  I   D  V  H
     L  I  S   V  V  M  R   T  L  -   R  P  S   R  R  A  S   M  C  T

121  CTTGACTGCC TCCAGGATCT ACAACGTAAA GCCAGAAGAA GTGAACGAAG AAATGCGACG
      L  D  C   L  Q  D   L  Q  R  K   A  R  R   S  E  R   R  N  A  T
→  T  L  T  A   S  R  I   Y  N  V   K  P  E  E   V  N  E   E  M  R
     P  -  L   P  P  G  S   T  T  -   S  Q  K   K  -  T   K  K  C  D

181  GGTTGGAAAG ATGGTGAACT TCTCTATAAT ATACGGTGTC ACACCGTACG GTCTTTCTGT
      G  W  K   D  G  E   L  L  Y  N   I  R  C   H  T  V   R  S  P  C
→  R  V  G  K   M  V  N   Ⓕ  S  I  I   Y  G  V   T  P  Y   G  L  S
     G  L  E   R  W  -  T   S  L  -   Y  T  V   S  H  R  T   V  F  L

241  GAGACTTGGA ATACCGGTTA AAGAAGCAGA AAAGATGATT ATCAGCTATT TCACACTGTA
      E  T  W   N  T  G   -  R  S  R   K  D  D   Y  Q  L   F  H  T  V
→  V  R  L  G   I  P  V   K  E  A   E  K  M  I   I  S  Y   F  T  L
     -  D  L   E  Y  R  L   K  K  Q   K  R  -   L  S  A  I   S  H  C

301  TCCAAAGGTG CGAAGCTACA TCCAGCAGGT TGTTGCAGAG GCAAAAGAGA AGGGCTACGT
      S  K  G   A  K  L   H  P  A  G   C  C  R   G  K  R   E  G  L  R
→  Y  P  K  V   R  S  Y   I  Q  Q   V  V  A  E   A  K  E   K  G  Y
     I  Q  R   C  E  A  T   S  S  R   L  L  Q   R  Q  K  R   R  A  T

361  CAGGACTCTC TTTGGAAGAA AAAGAGATAT TCCCCAGCTC ATGGCAAGGG ACAAGAACAC
      Q  D  S   L  W  K   K  K  R  Y   S  P  A   H  G  K   G  Q  E  H
→  V  R  T  L   F  G  R   K  R  D   I  P  Q  L   M  A  R   D  K  N
     S  G  L   S  L  E  E   K  E  I   F  P  S   S  W  Q  G   T  R  T

421  CCAGTCCGAA GGCGAAAGAA TCGCAATAAA CACCCCCATT CAGGGAACTG CGGCAGATAT
      P  V  R   R  R  K   N  R  N  K   H  P  H   S  G  N   C  G  R  Y
→  T  Q  S  E   G  E  R   I  A  I   N  T  P  I   Q  G  T   A  A  D
     P  S  P   K  A  K  E   S  Q  -   T  P  P   F  R  E  L   R  Q  I

481  AATAAAATTG GCTATGATAG ATATAGACGA GGAGCTGAGA AAAAGAAACA TGAAATCCAG
      N  K  I   G  Y  D   R  Y  R  R   G  A  E   K  K  K   H  E  I  Q
→  I  I  K  L   A  M  I   D  I  D   E  E  L  R   K  R  N   M  K  S
     -  -  N   W  L  -  -   I  -   T  R  S  -   E  K  E  T   -  N  P

541  AATGATCATT CAGGTTCATG ACGAACTGGT CTTCGAGGTT CCCGATGAGG AAAAAGAAGA
      N  D  H   S  G  S   -  R  T  G   L  R  G   S  R  -   G  K  R  R
→  R  M  I  I   Q  V  H   D  E  L   V  F  E  V   P  D  E   E  K  E
     E  -  S   F  R  F  M   T  N  W   S  S  R   F  P  M  R   K  K  K

601  ACTAGTTGAT CTGGTGAAGA ACAAAATGAC AAATGTGGTG AAACTCTCTG TGCCTCTTGA
      T  S  -   S  G  E   E  Q  N  D   K  C  G   E  T  L   C  A  S  -
→  E  L  V  D   L  V  K   N  K  M   T  N  V  V   K  L  S   V  P  L
     N  -  L   I  W  -  R   T  K  -   Q  M  W   -  N  S  L   C  L  L
```
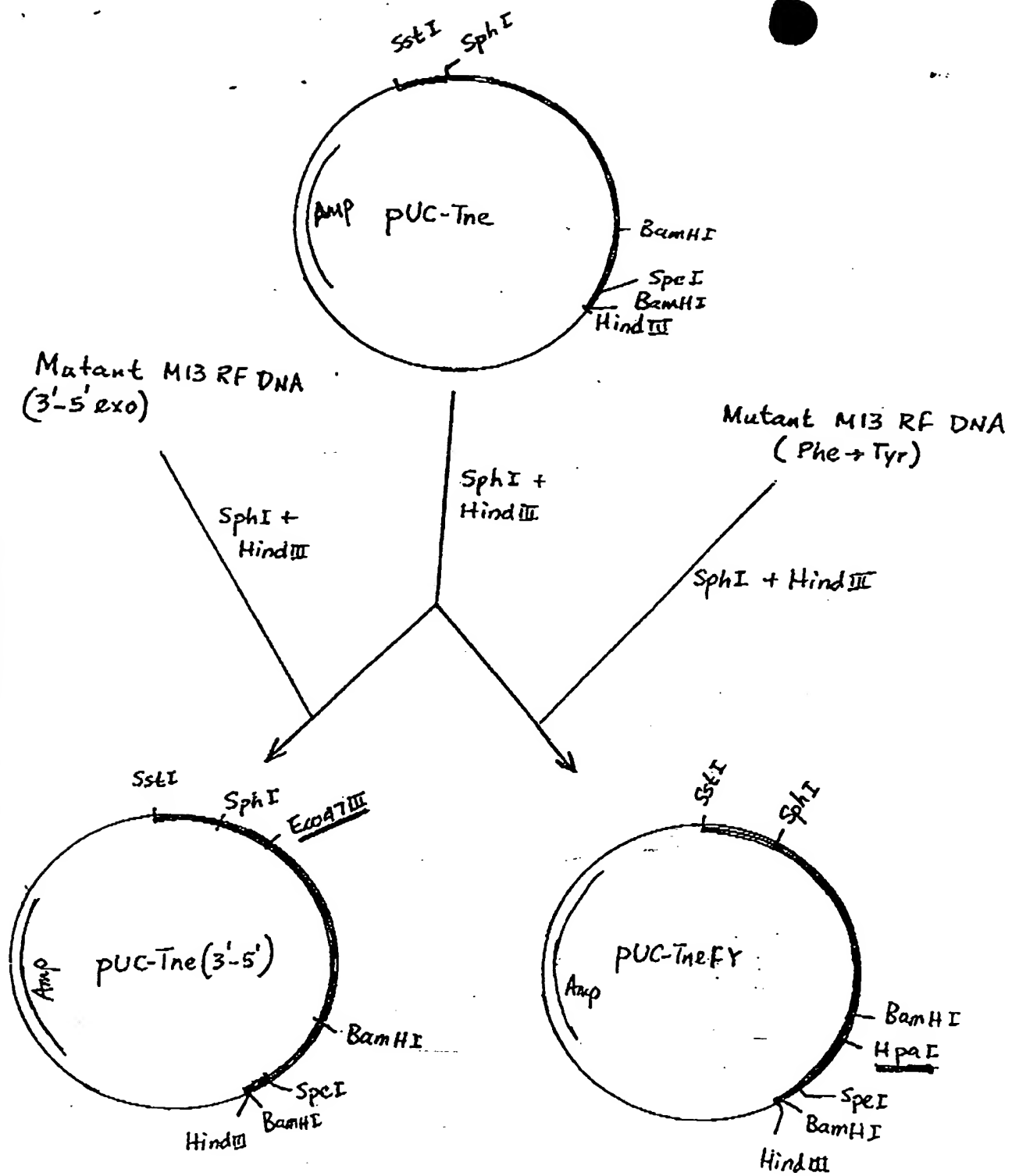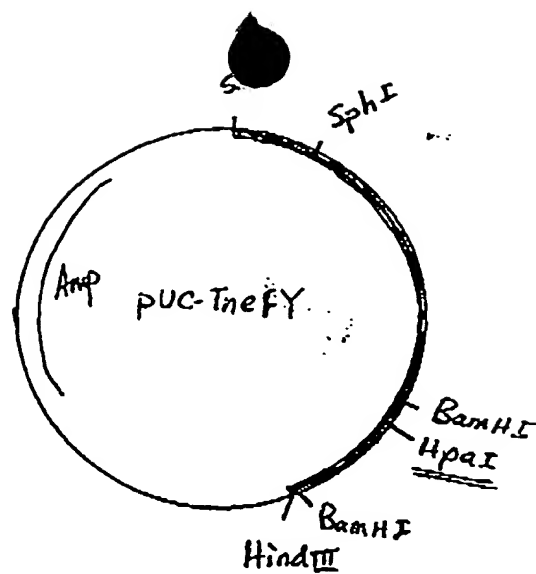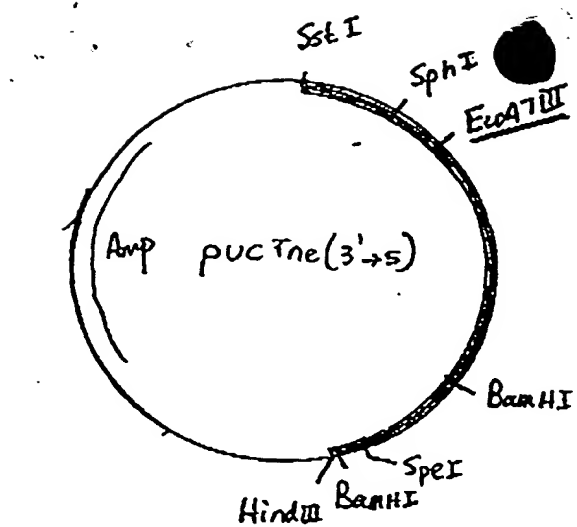
Fig 4

661 GGTTGACATA AGCATCGGAA A   GTC TTGA
     G - H   K H R   K   L V L
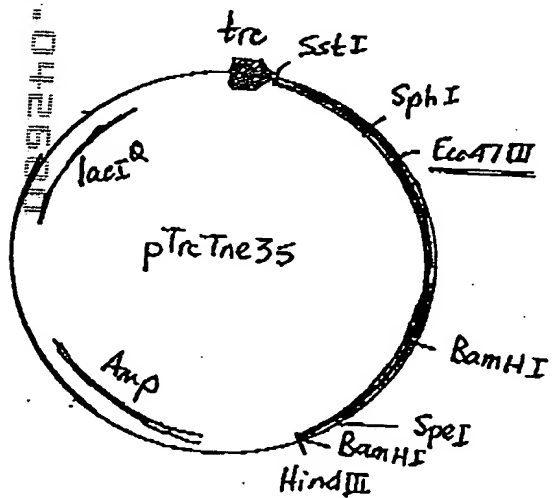→   E V D I S I G   K   S W S -
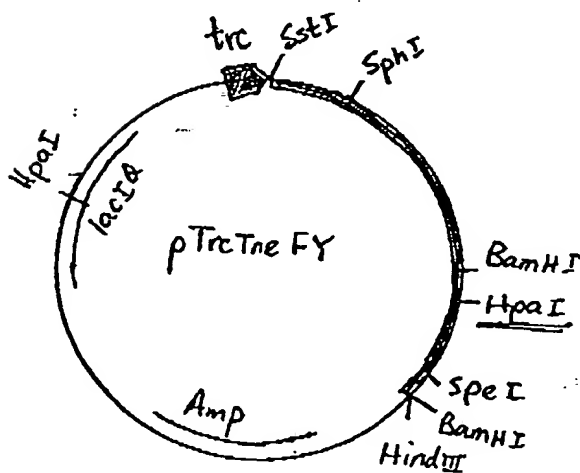   R L T -   A S E   K   A G L

Fig. 3A

Fig 3B

Fig A 6
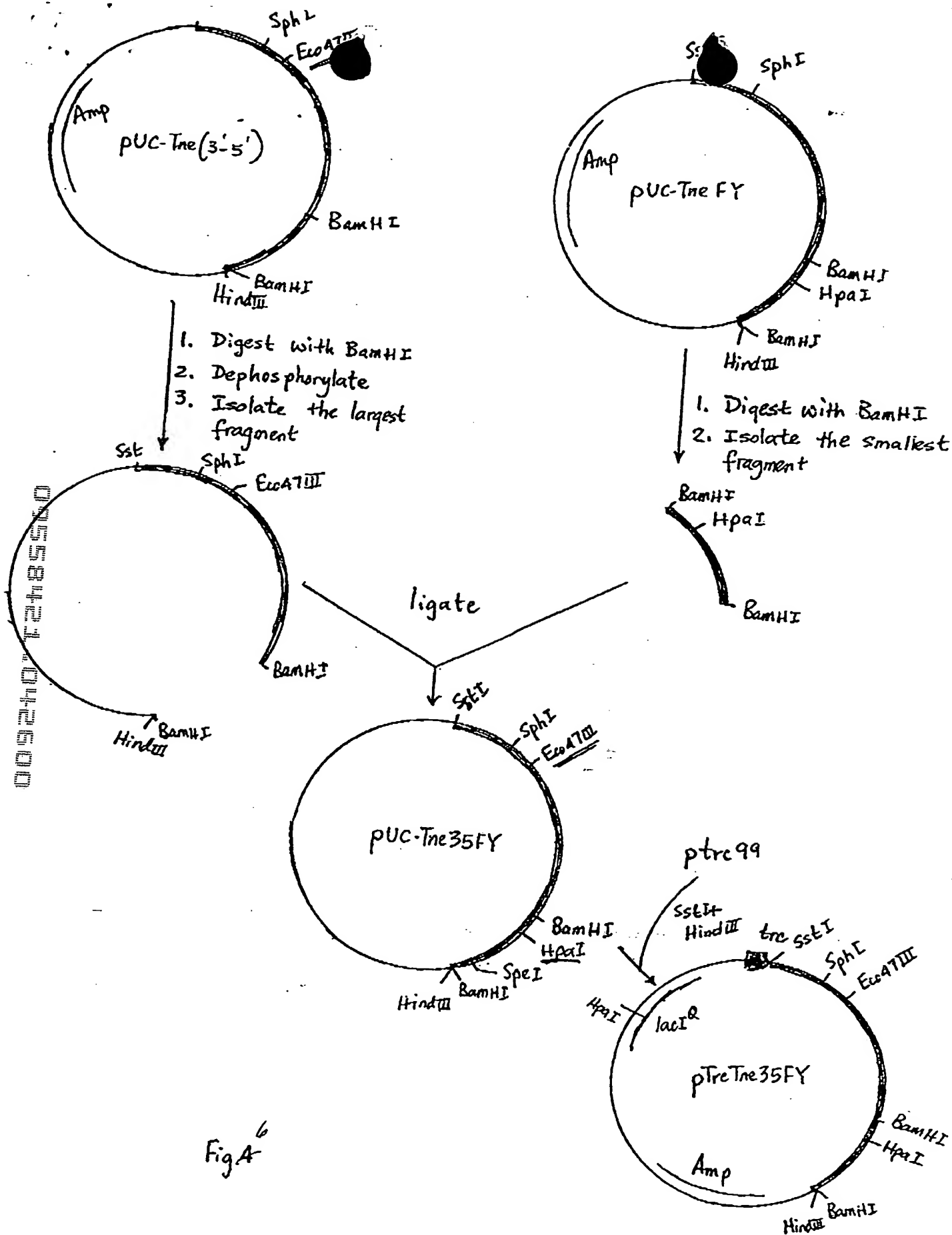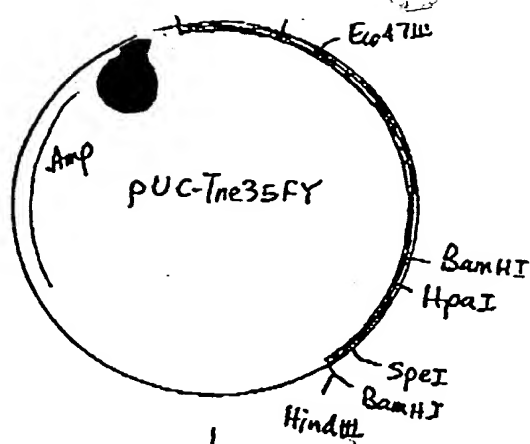
pUC-Tne35FY
- Amp
- Eco47III
- BamHI
- HpaI
- SpeI
- BamHI
- HindIII

1. Digest with HindIII
2. Fill in
3. Digest with SphI
4. Isolate Tne pol. fragment

pTTQ19 — SphI + SmaI

pTTQTne535FY
- tac
- SphI
- Eco47III
- BamHI
- HpaI
- SpeI
- BamHI

pUC-TneFY
- SstI
- SphI
- Amp
- BamHI
- HpaI
- SpeI
- BamHI
- HindIII
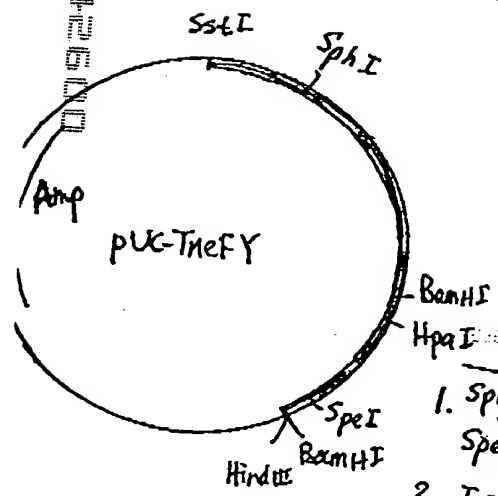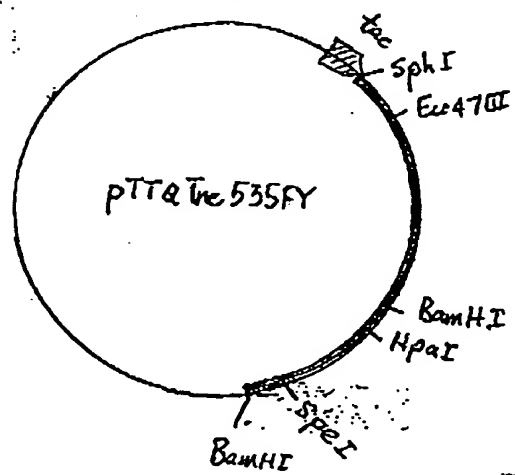
1. SphI + SpeI digestion
2. Isolate Vector plus small remaining Tne Pol fragment

1. SphI + SpeI digestion.
2. Isolate Tne Pol fragment

pTTQTne5FY
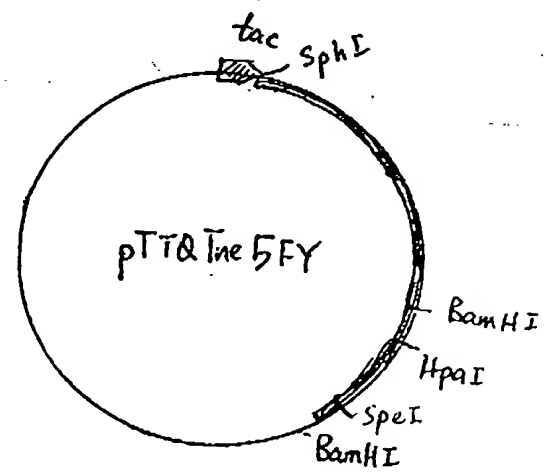- tac
- SphI
- BamHI
- HpaI
- SpeI
- BamHI

Fig. 7